Circulating cell-free DNA: The future of personalized medicine in ovarian cancer management

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Abstract

Objective: The objective of this study is to evaluate the utility of circulating cell-free DNA (cfDNA) in plasma to molecularly profile patients with ovarian high-grade serous carcinoma (HGSC).

Methods: Under IRB approval, we collected plasma and tumor from 14 patients with advanced HGSC before and after treatment with neoadjuvant chemotherapy (NACT). Plasma cfDNA and tumor DNA from each patient were subjected to next generation sequencing (NGS) utilizing a 50 cancer-related gene mutation panel. Mutations in cfDNA and tumor DNA specimens, both prior to and after NACT, were compared.

Results: Plasma cfDNA appears to detect more mutations in cancer related genes. Specifically, the cfDNA prior to NACT contained 19 mutated genes with 57 specific mutations; the tumor contained 6 mutated genes with 38 specific mutations (Table 2). Patients with mutations in 14 of the 19 genes could potentially benefit from FDA-approved targeted agents based on mutations in the cfDNA prior to NACT, compared to only five genes in the tumor. Similar findings were noted with respect to mutations in the cfDNA when compared to the tumor DNA after NACT. Specifically, 21 mutated genes with 54 mutations were identified in the cfDNA compared to 6 mutated genes and 37 mutations in the tumor. 18 of the 21 genes noted to be mutated in the cfDNA post NACT have FDA-approved targeted agents that could potentially be used, compared to only five genes in the tumor. Over the course of NACT, cfDNA showed more change in the genetic diversity. Specifically, of the 57 mutations in the plasma pre NACT, only 6 persisted after treatment, whereas 33 of 38 mutations in the tumor were the same after NACT.

Conclusions: Plasma cfDNA detects more mutations than DNA extracted from solid tumor and may better capture clonal evolution of genetic alterations. Precision medicine programs should consider utilizing cfDNA to optimize detection of the molecular diversity of ovarian cancer when performing genetic profiling.

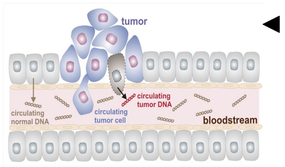

Figure 1. Circulating DNA in the Bloodstream. cfDNA can be shed from normal cells, circulating tumor cells, tumor cells or metastatic sites. The half-life of cfDNA is 15min to several hours.

Materials and Methods

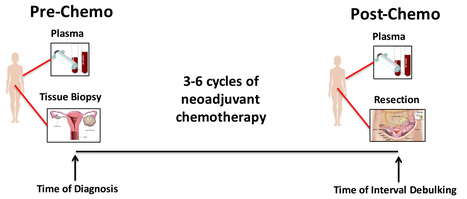

Figure 2. Schematic of Sample Collection. Tissue biopsy and blood was collected from 14 patients with advanced high grade papillary serous ovarian cancer at the time of diagnosis. Tumor and blood were collected at the time of the interval debulking following 3-6 cycles of neoadjuvant chemotherapy.

50 gene panel

ABL1	IDH2
AKT1	JAK2
ALK	JAK3
APC	KDR
ATM	KIT
BRAF	KRAS
CDH1	MET
CDKN2A	MLH1
CSF1R	MPL
CTNNB1	NOTCH1
EGFR	NPM1
ERBB2	NRAS
ERBB4	PDGFRA
EZH2	PIK3CA
FBXW7	PTEN
FGFR1	PTPN11
FGFR2	RB1
FGFR3	RET
FLT3	SMAD4
GNA11	SMARCB1
GNAQ	SMO
GNAS	SRC
HNF1A	STK11
HRAS	TP53
IDH1	VHL

Materials and Methods

- NGS was performed on 56 total samples (14 patients, 4 samples each)
- DNA was extracted from tissue using all prep-DNA Qiagen Kit
- cfDNA was isolated from plasma using Circulogene's proprietary cfDNA enrichment and recovery technology
- Quantification of cfDNA was performed using the Qubit 2.0
- Libraries were generated using the Ion AmpliSeq Library kit 2.0 and Cancer Hotspot Panel v2
- A 50-gene panel interrogating total ~3,000 mutations was performed on all samples using Ion Torrent Proton with coverage ranging from 3000-8000X
- Germline variants were screened out by excluding variants in the dbSNP
- Somatic variants were reported based on variants in COSMIC database
- Variants were called using Variant Caller 4.0 software
- All reported variants have 100X coverage, a quality score greater than 10, and a frequency higher than 1%
- Concordance was calculated using the following formula:

$$C = \frac{\text{Number of Concordant Pairs}}{\text{Number of Concordant Pairs + Number of Discordant Pairs}}$$

Results

Table 1. Demographics

Patient Characteristics	
Characteristics	Number of patients, (n=14)
Age at diagnosis (range)	Mean 73, Median 73.5 (61-85)
Stage	
IIIC	10
IV	4
Debulk Status	
NRD	5
Optimal	7
Suboptimal	2
Number of NeoAdj Cycles (range)	Mean 3.78, Median 4 (2-6)
CA-125	
Pre-NeoAdj	Mean 1038.9, Median 463.3 (79.3-5503)
Post-NeoAdj	Mean 277.56, Median 33.5 (4.7-2802)
Platinum Status	
Resistant/Refractory	5
Sensitive	7
Other (post op death/hospice)	2
Status after adjuvant therapy (n=12)	
Persistance	4
Complete Response	8
Overall Survival	Mean 15, Median 15.3 (4.3-25.4)
Progression Free Survival	Mean 11.3, Median 10.5 (4.8-18.4)
Mean Follow up	Mean 17.2, Median 16.6 (10.5-25.4)

Table 2. Total number of mutated genes/variants and Most frequently mutated genes

	cfDNA (plasma)		DNA (tumor)	
	Pre Chemo	Post Chemo	Pre Chemo	Post Chemo
# of mutated genes	19	21	6	6
# of variants	57	54	38	37
Most frequently mutated genes	P53 (20/57)	P53 (17/54)	P53 (23/38)	P53 (23/37)
	PIK3CA (6/57)	KIT (7/54)	KDR (5/38)	KDR (6/37)
	KIT (5/57)	PIK3CA (5/54)	KIT (5/38)	KIT (4/37)
	EGFR (4/57)	CTNNB1 (3/54)	PIK3CA (3/38)	PIK3CA (2/37)

	Pre-Chemo			Post-Chemo			Total cfDNA	
	Gene	Alteration	%	Gene	Alteration	%	Pre	Post
P-1	PIK3CA	I391M	100	PTPN11	D61G	4.9	197	148
	TP53	splice	6.3	TP53	E126G	2.9		
	TP53	G120E	3	PIK3CA	H1047Y	2.3		
T-1	PIK3CA	I391M	97.8	TP53	P33R	91		
	TP53	Y124C	83	TP53	splice	88		
P-2	PIK3CA	I391M	100	NRAS	G12D	20.1	164	98
	SMAD4	C499R	4.8	KIT	M537L	32.1		
	PTEN	K66E	2.6	FGFR2	K195R	12.4		
				ATM	L1322P	5.4		
				TP53	C110R	2.5		
T-2	TP53	R123G	60.5	TP53	R123G	59.1		
	KIT	M537L	23.2	KIT	M537	27.7		
P-3	TP53	P33R	55.4	TP53	P33R	96.1	95	104
	KDR	Q472H	47.4	KDR	Q472H	51.7		
	RET	G630R	3	FBXW7	H382R	3.6		
	KIT	W553G	2.5	TP53	M10T	2		
	TP53	K132R	2					
T-3	TP53	Y181C	70.2	KDR	Q472H	49.6		
	KDR	Q472H	37	TP53	P33R	44.5		
	TP53	P33R	13.9	TP53	Y181C	2.5		
P-4	TP53	P33R	97	ERBB2	Q765R	11.5	84.2	99.8
	VHL	S139P	42.6	EGFR	P596L	6.9		
	TP53	A117V	15.9	TP53	L167*	5.1		
	CTNNB1	A43T	5.4	PTEN	E284K	4.1		
	APC	A1452T	4.9	KIT	M537L	3.1		
	TP53	I123T	3.1	TP53	K132R	2.5		
				APC	I862T	2.2		
T-4	TP53	P33R	95.2	TP53	P33R	94.7		
				KDR	Q472H	6.7		
P-5	KIT	V565A	11.3	TP53	P33R	95.5	125	140
				KDR	Q472H	18.4		
				KIT	K546R	7.4		
				RET	E632K	4.9		
				GNAS	R216H	3.8		
				CTNNB1	A20V	2.5		
				PTEN	D331G	2.1		
T-5	KDR	Q472H	53.9	KDR	Q472H	52.5		
	TP53	P33R	34.2	TP53	P33R	50.7		
	TP53	R117G	18.3	TP53	R117G	2		
P-6	APC	K1332*	58.5	KIT	M537L	70.9	110	134
	KIT	M537L	53.6	TP53	M114T	7.7		
	TP53	P33R	13.3	TP53	I122T	7.5		
	PIK3CA	M1043V	5.2	ATM	Y1753C	4.9		
	BRAF	F595L	2.7	EGFR	H870Y	3.3		
				TP53	Y124H	3		
				KIT	K554R	2.7		
				TP53	M105L	2.4		
T-6	KIT	M537L	53.2	KIT	M537L	51.2		
	TP53	P33R	36	TP53	P33R	36.4		
	TP53	SPLICE	15.5	TP53	SPLICE	22.6		
P-7	EGFR	T785A	9.2	No Mut. Detected			133	88
	EFGR	R108G	7.4					
	TP53	K132R	2.6					
	TP53	R131C	2.2					
	TP53	D169G	1.9					
T-7	TP53	R114C	4	TP53	R114C	40.6		

	Pre-Chemo			Post-Chemo			Total cfDNA	
	Gene	Alteration	%	Gene	Alteration	%	Pre	Post
P-8	TP53	P33R	69.7	PIK3CA	S326F	59.8	116	126
				PIK3CA	F909L	6.2		
T-8	PTEN	R15G	31.8	TP53	P33R	39.8		
	TP53	S144*	29.7	TP53	S144*	14		
	TP53	P33R	29	PTEN	R15G	13		
P-9	IDH1	I102T	13.5	BRAF	S607P	3.8	105	120
	GNAQ	S225P	6.8					
	FGFR2	V277A	3.7					
T-9	KDR	Q472H	98.8	KDR	Q472H	98.6		
	TP53	P33R	73.2	TP53	P33R	84.2		
	KIT	M537L	64.5	TP53	Y124S	81		
	TP53	Y124S	56.6	KIT	M537L	70.5		
P-10	TP53	P33R	94.5	TP53	Y126C	68.2	102	268
	TP53	V158A	5.7	APC	Q1411*	4.3		
	KDR	Q472H	5	TP53	K132E	3		
	APC	S1337P	4.1					
	TP53	F109S	3.7					
T-10	KRAS	G12S	28.4	KRAS	G12S	32		
	TP53	P33R	93.4	TP53	P33R	93.7		
	KDR	Q472H	5.5	KDR	Q472H	5.7		
P-11	TP53	P33R	95.2	PIK3CA	I391M	59.7	71.6	102
	PIK3CA	I391M	64.9	CTNNB1	I35T	2.7		
	PTEN	Y178C	5					
	ERBB4	Y268C	4.8					
	TP53	L155P	3.4					
	EGFR	F712L	2.3					
T-11	TP53	P33R	94	TP53	P33R	92.4		
	TP53	D122G	78.7	PIK3CA	I391M	40.1		
	PIK3CA	I391M	9.5					
P-12	KDR	Q472H	98.2	GNAQ	Q209R	3.1	128	118
	TP53	M114T	9.8	MET	H1094R	2.8		
	ATM	L2946S	5.4	BRAF	K601E	2.4		
	TP53	F111S	3.9					
	KIT	K638E	3.4					
	SMARCB1	R40*	3.2					
	EGFR	K745R	3.1					
T-12	TP53	P33R	95.1	TP53	P33R	92.6		
	TP53	V177M	79.8	TP53	V177M	83		
	KDR	Q472H	66	KDR	Q472H	65		
P-13	PIK3CA	I391M	100	PIK3CA	I391M	100	100	81
	TP53	I100T	4.2	TP53	P33R	96.1		
				TP53	R183*	19.1		
				TP53	L130P	9.1		
				KIT	K546R	5.4		
T-13	PIK3CA	I391M	59.7	PIK3CA	I391M	97.8		
	TP53	P33R	33.1	TP53	P33R	57		
	TP53	R123G	30.9	TP53	I156T	25.7		
	TP53	I156T	7.3					
	KIT	M537L	2.1					
P-14	APC	Q1349*	45.2	TP53	P33R	97.6	116	83
	KIT	M537L	27.8	KIT	M537L	63.3		
	PTEN	E291K	18.2	JAK2	L609S	29.9		
	ABL1	M351V	5.1	CTNNB1	S45P	6.7		
	PIK3CA	M1040V	4.5					
T-14	TP53	P33R	94.6	TP53	P33R	94.2		
	KIT	M537L	52.2	KIT	M537L	51.7		
				TP53	R183P	15.1		

Results

Table 3 . Frequency of each variant and total cfDNA pre and post chemotherapy. Specific variants for each gene that is mutated in all 14 patients is shown pre (green) and post (blue) chemotherapy in both the plasma (P) (light green/blue) and tumor (T) (dark green/blue). Total amount of cfDNA (ng/μL) in the plasma is shown (purple) pre and post chemotherapy

Gene	freq in Tissue	freq in Plasma	Overall Concordance
TP53	100	68	36
KIT	35	39	64
PTEN	7	18	75
PIK3CA	21	36	79
CTNNB1	0	14	86
BRAF	0	11	89
EGFR	0	11	89
APC	0	7	93
ATM	0	7	93
FGFR2	0	7	93
GNAQ	0	7	93
KDR	7	7	93
RET	0	7	93
KRAS	7	0	93
ABL1	0	4	96
ERBB2	0	4	96
ERBB4	0	4	96
FBXW7	0	4	96
GNAS	0	4	96
IDH1	0	4	96
JAK2	0	4	96
MET	0	4	96
NRAS	0	4	96
PTPN11	0	4	96
SMAD4	0	4	96
SMARCB1	0	4	96
VHL	0	4	96

FDA approved therapy	Clinical Trials
NA	WEE1 inhibitors
Tyrosine kinase inhibitors-imatinib, nilotinib, sorafenib, dasatinib, sunitinib	Tyrosine kinase inhibitors
MTOR inhibitors- everolimus, temsirolimus; Parp inhibitor-olaparib	MTOR/Parp inhibitors
MTOR inhibitors- everolimus, temsirolimus	MTOR inhibitors
MTOR inhibitors- everolimus, temsirolimus	WNT inhibitors
BRAF inhibitors-vemurafenib, dabrafenib; MEK inhibitors-tametinib, cobimentinib	MEK inhibitors
lapatinib, erlotinib, gefitinib	EGFR inhibitors
NA	WNT inhibitors
Parp inhibitor- olaparib	ATM inhibitors
Multikinase inhibitors -pazopanib, ponatinib	Multikinase inhibitors
MEK inhibitors-tametinib, cobimentinib	PKC inhibitors
Cabozantinib, axitinib, sorafenib, regorafenib, sunitinib	Anti angiogenics
Multikinase inhibitors – vandetanib, ponatinib, sunitinib	RET inhibitors
MEK inhibitors – trametinib, cobimentinib	MEK inhibitors
ABL inhibitor-imatinib, nilotinib, dasatinib, bosutinib, ponatinib	Tyrosine kinase inhibitors
lapatinib, afatnib, neratinib, dacomitinib, ado-trastuzumab, emtansine, pertuzumab	HER2 inhibitors
Tyrosine kinase inhibitors – gefitinib, lapatinib	Pan-Her inhibitors
MTOR inhibitors - everolimus , temsirolimus	MTOR inhibitors
MEK inhibitors – trametinib, cobimentinib	ERK inhibitors
NA	IDH1 inhibitors
NA	JAK2 inhibitors
MET inhibitors- crizotinib, cabozantinib	MET inhibitors
BRAF inhibitors-vemurafenib, dabrafenib; MEK inhibitors-tametinib, cobimentinib	MAP/ERK inhibitors
NA	PTPN11/SHP2 inhibitors
NA	TGFβ inhibitors, anti angiogenics
NA	EZH2 inhibitors
Tyrosine kinase inhibitors – sunitinib, sorafenib, MTOR inhibitors – everolimus, temsirolimus	VEGF inhibitors

Table 4 . Frequency of alterations, overall concordance, and potential targeted therapy. The frequency (%) of each mutation of the genes with at least one mutation in the plasma and/or tumor are shown.

Conclusions

- Mutations are more frequently detected in the plasma compared to the tumor
- Tumor heterogeneity is better captured by circulating cfDNA than from a small biopsy of tumor
- More mutation changes are picked up in the plasma following therapy
- Caution must be taken in interpreting the results for clinical purposes because much is still unknown about the dynamic biology of circulating cfDNA
- One theory is that cfDNA represents mainly the genome of dying tumor cells; therefore, mutations in this population of DNA should NOT be used to guide targeted therapy because DNA in viable tumor cells contain the mutations that drive cancer progression and cause therapy resistance
- We need more research and clinical trials to investigate the utility of a "liquid biopsy"

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